Helsinki, 7 July 2017

Substance name: di-tert-pentyl peroxide (DTA)
EC number: 234-042-8
CAS number: 10508-09-5
Date of Latest submission(s) considered[1]: 22 September 2015
Decision/annotation number: Please refer to the REACH-IT message which delivered this communication (in format SEV-D-XXXXXXXXXX-XX-XX/F)
Addressees: Registrant(s)[2] of di-tert-pentyl peroxide

DECISION ON SUBSTANCE EVALUATION

1. Requested information

Based on Article 46(1) of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), you are requested to submit the following information on the registered substance:

1.1 *In vitro* mammalian cell micronucleus test with fluorescence *in situ* hybridisation (FISH) or immunochemical labelling of kinetochores (CREST) (OECD 487/EU B.49)
1.2 A more detailed justification for the read-across, as specified under section "the concern(s) identified" of Appendix 1.

You shall provide an update of the registration dossier(s) containing the requested information, including robust study summaries and, where relevant, an update of the Chemical Safety Report by **15 October 2018**. The deadline takes into account the time that you, the Registrant(s), may need to agree on who is to perform any required tests.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2. Further information, observations and technical guidance as appropriate are provided in Appendix 3. Appendix 4 contains a list of registration numbers for the addressees of this decision. This appendix is confidential and not included in the public version of this decision.

2. Appeal

You can appeal this decision to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, shall be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under http://echa.europa.eu/regulations/appeals

Authorised[3] by Claudio Carlon, Head of Unit Evaluation 2

[1] This decision is based on the registration dossier(s) at the end of the 12 month evaluation period.

[2] The terms Registrant(s), dossier(s) or registration(s) are used throughout the decision, irrespective of the number of registrants addressed by the decision.

[3] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

EUROPEAN CHEMICALS AGENCY

Appendix 1: Reasons

Based on the evaluation of all relevant information submitted on di-tert-pentyl peroxide (DTA) and other relevant available information, ECHA concludes that further information is required in order to enable the evaluating Member State Competent Authority (eMSCA) to complete the evaluation of whether the substance constitutes a risk to human health. The eMSCA will subsequently review the information you submit and evaluate if further information should be requested in order to clarify the concern for mutagenicity and carcinogenicity.

1.1 Mutagenicity and 1.2 read-across

The concern(s) identified

The substance was tested for genotoxicity in the *in vitro* and *in vivo* tests. No positive results were observed for gene mutation both in bacterial and in cell systems. No chromosomal aberration was observed in human lymphocytes *in vitro*.

Positive results in a micronucleous test *in vivo* in mice treated intraperitoneally was obtained. No data are available for the registered substance in the germ cells mutation but you presented a read-across with the analogue substance di-tert-butyl peroxide (DTB). Negative results were observed in the *in vivo* chromosomal aberration assay in spermatogonial germ cell in mice after intraperitoneal administration.

DTB was evaluated by ECHA's Committee for Risk Assessment (RAC) and as reported in the opinion of 27 January 2010, the substance has a harmonised classification as "mutagen category 2" under the CLP Regulation (Regulation (EC) No 1272/2008) on the basis of the available information. Applying the read-across, you self-classified DTA as "mutagen category 2".

As reported in the justification document the toxicological mode of action of DTA and DTB are presumed to be related to the peroxide group, the substance itself and the potential metabolism byproducts. ECHA notes that you have only used general statements as an attempt to justify why the available data can be used to predict the genotoxicity in germ cells. The available information is not sufficient to rule out a role of a different genotoxic potential of the substance itself of a different potential metabolism byproducts of the two substances.

In particular, eMSCA has made the following observations regarding your read-across argumentation:

(i) No data and poor reasoning on the metabolism and excretion of DTA and DTB are provided:

You state: "it is suspected that both substances will be enzymatically hydrolysed, …". eMSCA notes that the possible metabolism is only sketched, i.e. not exhaustive of all possible metabolites (e.g. possibility of radicals generation) and not adequately reasoned (e.g. references inadequate).

(ii) The mechanism of toxicity of the two substances is not adequately discussed:

You state: "The common functional group is R-O-O-R…". eMSCA notes that the possible mechanisms of toxicity, known to be related to the abovementioned

functional group, are not discussed. In particular, modes of action of importance for genotoxicity/carcinogenicity mechanisms, should have been investigated.

(iii) Differences in the toxicological behaviour of DTA and DTB are not thoroughly discussed:

In particular, eMSCA notes a difference in skin and eye irritation potential. The significance of this behaviour should be discussed, both in mechanistic terms and with regard to the possible relevance for other endpoints.

Structural similarity is a prerequisite for applying the grouping and read-across approach, but does not necessarily lead to predictable or similar human health properties. It has to be established why and how predictions based on grouping and read-across are reliable. In view of the uncertainties described (lacking clarity on mechanism, no information on kinetics/metabolism, possible differences in toxicity) the read-across justification needs to be strengthened and clarified. A comparative assessment or investigation if needed on the kinetics/metabolism of source and target substance and on the reactivity of these substances towards biological macromolecules (i.e. DNA, proteins) would provide better insight in the reliability of the proposed prediction.

The *in vivo* positive result of DTA in the bone marrow by intraperitoneal route also raises a concern on genotoxicity as a potential mode of action for carcinogenicity, in particular locally at the site of contact.

Data from other structurally-close peroxides may exist in relation to this concern and may contribute to the overall understanding of the genotoxicity profile of DTA and its carcinogenic potential.

In this purpose, the inclusion and justification of other structurally-close peroxides in the read-across assessment may also be considered, if relevant, in the aim to limit the possible need to require further testing on DTA to answer that concern.

For these reasons, the acceptance of the read-across for genotoxicity on germ cells could lead to an underestimation of the hazard of the non-tested substance (DTA). Therefore, in order to proceed with an appropriate *in vivo* follow-up, it is necessary to understand the mechanistic basis of the results observed *in vitro.*

Why new information is needed

ECHA is of the opinion that the available information is insufficient to drawn a final conclusion about mutagenicity of DTA. In order to clarify this issue, an *in vitro* micronucleus test with centromere detection is warranted (e.g. fluorescence *in situ* hybridisation (FISH) analysis or immunochemical detection of kinetochores (CREST)), i.e. with the possibility to discriminate between clastogenic and aneugenic effects.

Moreover, the read-across justification document is not acceptable in the current form. As a baseline, despite the structural differences no appropriate reason is provided why and how a prediction can be made, neither for genotoxic properties nor for the other high tier human health properties. The main reason mentioned is structural similarity, which is not acceptable per se.

The eMSCA also evaluated the data presented on the analogue substance DTB, but

concluded that also these data are indicative of a possible aneugenic mechanism of the substance. In fact, DTB is also under substance evaluation by the Netherlands as indicated in the Community rolling action plan (CoRAP) 2016. The failure of the appropriateness of the read-across could trigger a request for the missing *in vivo* test on DTA.
An important distinction should be made between the Risk Assessment Committee (RAC) process for harmonised classification and labelling and the substance evaluationin Member State Committee (MSC): the goal of the latter is to clarify the identified concern, by requesting additional information, while the RAC opinion is based only on the available data

Considerations on the test method and testing strategy

The *in vitro* micronucleus assay is a genotoxicity test for the detection of micronuclei in the cytoplasm of interphase cells. Micronuclei may originate from acentric chromosome fragments (i.e. lacking a centromere), or whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell division. The assay detects the activity of clastogenic and aneugenic chemicals in cells that have undergone cell division during or after exposure to the test substance (OECD 487/EU B.49). ECHA deems that this assay will clarify the mechanism of genotoxicicity of the test substance. The results of this assay will be used to orientate the further experimental strategy including the possibility to request new data on germ cells or on carcinogenicity.

Moreover, the failure of the appropriateness of the read-across could trigger a request of further *in vivo* testing on DTA to clarify the concern for germ cell mutagenicity and if adequate risk management measures have to be taken (e.g. classification proposal).

Alternative approaches and proportionality of the request

You may adapt the testing requested above according to the specific rules outlined in Annexes VI to X and/or according to the general rules contained in Annex XI of the REACH Regulation. In order to ensure compliance with the respective information requirement, any such adaptation will need to have a scientific justification, referring to and conforming with the appropriate rules in the respective Annex, and an adequate and reliable documentation.

Conclusion

Therefore, based on the substance evaluation and pursuant to Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following study using the registered substance subject to this decision: *in vitro* mammalian cell micronucleus test with fluorescence *in situ* hybridisation (FISH) or immunochemical labelling of kinetochores (CREST) (OECD 487/EU B.49); and provide a more detailed justification for the read-across, as specified above under section "the concern(s) identified".

Appendix 2: Procedural history

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to suspected C, suspected M, wide dispersive use, exposure of workers, di-tert-pentyl peroxide, CAS No 10508-09-5 (EC No 234-042-8) was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2015. The updated CoRAP was published on the ECHA website on 17 March 2015. The Competent Authority of Italy (hereafter called the evaluating MSCA) was appointed to carry out the evaluation.

Pursuant to Article 45(4) of the REACH Regulation the evaluating MSCA carried out the evaluation of the above substance based on the information in your registration(s) and other relevant and available information.

The evaluating MSCA considered that further information was required to clarify the concern for mutagenicity and in a step-wise approach the concern for carcinogenicity. Therefore, it prepared a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information. It submitted the draft decision to ECHA on 17 March 2016.

Registrant(s)' commenting phase

On 26 April 2016, ECHA sent the draft decision to you and invited you pursuant to Article 50(1) of the REACH Regulation to provide comments within 30 days of the receipt of the draft decision. This deadline includes an extra seven-day period as addressed in the last update point 9(d) of the Terms of Conditions of REACH-IT.

On 2 June 2016, you submitted your comments to ECHA.

Proposals for amendment by other MSCAs and ECHA and referral to Member State Committee

The evaluating MSCA notified the draft decision to the Competent Authorities of the other Member States and ECHA for proposal(s) for amendment.

Subsequently, the evaluating MSCA received proposal(s) for amendment to the draft decision and modified the draft decision. They are reflected in the Reasons (Appendix 1).

ECHA referred the draft decision, together with your comments, to the Member State Committee.

ECHA invited you to comment on the proposed amendment(s).

Your comments on the proposed amendment(s) were taken into account by the Member State Committee and are reflected in the Reasons (Appendix 1).

MSC agreement seeking stage

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-54 meeting and ECHA took the decision according to Article 52(2) and 51(6) of the REACH Regulation.

The decision making followed the procedure of Articles 50 and 52 of the REACH

Regulation.

Appendix 3: Further information, observations and technical guidance

1. This decision does not imply that the information provided by you in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on your dossier(s) at a later stage, nor does it prevent a subsequent decision under the current substance evaluation or a new substance evaluation process once the present substance evaluation has been completed.

2. Failure to comply with the request(s) in this decision, or to fulfil otherwise the information requirement(s) with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State.

3. In relation to the required experimental study/ies, the sample of the substance to be used shall have a composition that is within the specifications of the substance composition that are given by all Registrant(s). It is the responsibility of all the Registrant(s) to agree on the tested material to be subjected to the test(s) subject to this decision and to document the necessary information on composition of the test material. The substance identity information of the registered substance and of the sample tested must enable the evaluating MSCA and ECHA to confirm the relevance of the testing for the substance subject to substance evaluation.